



*Original article*

## Physiological mechanism involved in St. John's wort (*Hypericum perforatum* L.) response to salinity and effect of ascorbic acid foliar application

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### *Extended abstract*

#### **Introduction**

St John's wort is a valuable medicinal plant with treating depression and wound-healing. It's belonging to the Hypericaceae family that are rich in polyphenols. Hyperforin and Hypericin are two biological active substances of this species that have anticancer properties. In regard to the pharmaceutical properties of this plant, the demand for St John's wort products has been increased. Therefore it's required to investigate plant adaption to different growing condition such as salinity. Scarcity of water resources, soil and water salinity are concern of 21st century. Salinity is the main widespread water quality problem for crop production. Population growth, rapid urbanization, climate changes and lowering of the rainfall are the main causes of salinization of water and land. One way to reduce adverse effects of salt in salt affected areas is using compounds such as antioxidant. Ascorbic acid (AA) is the most abundant and small antioxidant molecule. It has been participating in ROS scavenging and led to broad tolerance to salt. Understanding the physiological and biochemical mechanism of St John's wort to salinity will be crucial for growing plants on salt affected soils or irrigated with saline water in the big scale.

#### **Materials and methods**

The pot experiment was done in factorial scheme in a completely randomized design with 3 replications during 2016 and 2017 spring season. In this experiment, St john's wort plant (Hungarian variety) were treated by different saline water concentrations including 2 (control), 6 and 10 dS/m and ascorbic acid foliar application (0 (control), 200, 400 mg/l). Treatments were started at 12 leaves stage (10 cm height). Foliar spray was carried out 2 times by 7 day interval. Salinity treatments were started 7 days after second spraying. The plants were harvested 30 days after the beginning of salinity treatments. Photosynthesis pigments of leaf, total phenols, hydrogen peroxide, cations (Na and K) and dry weight of shoot and root were measured.

#### **Results and discussion**

Based on results, salinity decreased photosynthesis pigments, carotenoid, leaf phenol, root potassium, K/Na Ratio in both leaf and root as well as shoot and root dry weights. But with increasing NaCl,

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hydrogen peroxide and sodium in both leaf and root parts increased. One of the reasons for the reduction in chlorophyll and carotenoids content is oxygen radicals which degrade chlorophyll. Phenolic compounds can play a critical role during salinity stress by minimizing the ionic imbalance caused by the presence of salts which in turn has a positive role in water absorption and balance in the plant. They have strong antioxidant activities, therefore may be used in defense against ROS that produces under stress. No significant changes found in root phenol and leaf potassium compared to the control. Unchanged potassium content in the leaves showed the roots ability to retain potassium as an important salt tolerance mechanism. Stomatal pore can be closed by the accumulation of sodium and chloride, reduced carbon dioxide fixation and plant growth reduction. Ascorbic acid application increased, Chl a, Total Chl, carotenoid, leaf phenol, leaf potassium and sodium, shoot and root dry weights compared to control and decreased leaf and root hydrogen peroxide. During stress condition, Abscisic acid increases hydrogen peroxide which induces stomatal closure; while application of ascorbic acid reversed this action. In plants that treated with ascorbic acid and salinity, Chl a, leaf phenol, leaf and root hydrogen peroxide, leaf potassium, root K/ Na ratio, shoot and root dry weights reduced less compared to the plants which only treated with salinity.

### Conclusions

Based on the results of this experiment increase in phenolic compounds due to their antioxidants properties, maintaining leaf potassium are possible salt tolerance mechanisms. The highest concentration of ascorbic acid (400 mg/l) was more effective in minimizing negative effects of salinity.

**Keywords:** Ascorbic acid, Oxygen reactive species, Photosynthetic pigments, Salinity tolerance, St John's wort

**Table 1. Results of variance analysis for the effects of salinity levels and ascorbic acid foliar application on pigment amounts of St John's wort**

S.O.V	df	Chl a	Chl b	Total Chl	Carotenoids
Salinity (S)	2	0.199**	0.010**	0.179**	0.018**
Foliar application (F)	2	0.040**	0.002 <sup>ns</sup>	0.128**	0.010*
S × f	4	0.066**	0.004 <sup>ns</sup>	0.098 <sup>ns</sup>	0.011 <sup>ns</sup>
Error	9	0.010	0.005	0.068	0.009
CV%		11	26	23	22

ns: Non-significant, \*\*, \*:significant at 1% and 5% probability level respectively

**Table 2. Mean comparison of salinity levels and ascorbic acid foliar application on pigments of St John's wort**

Treatments	Chl a	Chl b	Total Chl	Carotenoids
-----Mg/g FW-----				
<b>Salinity levels (dS.m<sup>-1</sup>)</b>				
2	*0.43 <sup>a</sup>	0.12 <sup>a</sup>	0.51 <sup>a</sup>	0.19 <sup>a</sup>
6	0.28 <sup>b</sup>	0.08 <sup>ab</sup>	0.35 <sup>ab</sup>	0.14 <sup>ab</sup>
10	0.18 <sup>c</sup>	0.06 <sup>b</sup>	0.27 <sup>b</sup>	0.11 <sup>b</sup>
<b>Ascorbic acid foliar application (mg/L)</b>				
0	0.24 <sup>b</sup>	0.07 <sup>a</sup>	0.29 <sup>b</sup>	0.12 <sup>b</sup>
200	0.35 <sup>a</sup>	0.10 <sup>a</sup>	0.49 <sup>a</sup>	0.18 <sup>a</sup>
400	0.30 <sup>ab</sup>	0.08 <sup>a</sup>	0.35 <sup>ab</sup>	0.13 <sup>b</sup>

In each column and for each treatment means with the same letter do not have statistically significant differences at 5% level of probability according to LSD

**Table 3. Results of variance analysis for the effect of salinity and ascorbic acid foliar application on phenol and hydrogen peroxide contents of leaf and root of St John's wort**

S.O.V	df	Leaf total phenol	Root total phenol	Leaf H <sub>2</sub> O <sub>2</sub>	Root H <sub>2</sub> O <sub>2</sub>
Salinity (S)	2	741.42**	24.14 <sup>ns</sup>	3208**	90.41**
Foliar application (F)	2	873.67**	84.28 <sup>ns</sup>	1252*	108.80**
S × F	4	248.23*	268.24 <sup>ns</sup>	3200**	239.09**
Error	9	181.88	206.54	1096	34.15
CV%		4	8	8	11

ns: Non-significant, \*\*, \*:significant at 1% and 5% probability level respectively.

**Table 4. Mean comparison of salinity levels and ascorbic acid foliar application on phenol and hydrogen peroxide contents of leaf and root of St John's wort**

Treatments	Leaf total phenol	Root total phenol	Leaf H <sub>2</sub> O <sub>2</sub>	Root H <sub>2</sub> O <sub>2</sub>
<b>Salinity levels (dS.m<sup>-1</sup>)</b>	----- (mg GA/g FW) -----		-----μmol/ g FW-----	
2	*106.88 <sup>b</sup>	58.96 <sup>a</sup>	115.58 <sup>b</sup>	14.41 <sup>b</sup>
6	109.71 <sup>b</sup>	59.46 <sup>a</sup>	127.43 <sup>ab</sup>	16.48 <sup>ab</sup>
10	121.68 <sup>a</sup>	61.43	147.90 <sup>a</sup>	19.85 <sup>a</sup>
<b>Ascorbic acid foliar application (mg.L<sup>-1</sup>)</b>				
0	105.19 <sup>b</sup>	57.39 <sup>a</sup>	138.56 <sup>a</sup>	19.93 <sup>a</sup>
200	122.00 <sup>a</sup>	59.51	118.87 <sup>b</sup>	13.91 <sup>b</sup>
400	111.07 <sup>a</sup>	62.66	133.48 <sup>a</sup>	16.91 <sup>ab</sup>

In each column and for each treatment means with the same letter do not have statistically significant differences at 5% level of probability according to LSD

**Table 5 Results of variance analysis for the effect of salinity and ascorbic acid foliar application on potassium, sodium and K/Na ratio of leaf and root of St John's wort**

S.O.V	df	Leaf potassium	Root potassium	Leaf sodium	Root sodium	Leaf K/Na Ratio	Root K/Na Ratio
Salinity (S)	2	8576 <sup>ns</sup>	28048*	507140**	66128**	0.13**	0.10**
Foliar application(F)	2	19130*	2048 <sup>ns</sup>	91369 <sup>ns</sup>	51282**	0.06 <sup>ns</sup>	0.01 <sup>ns</sup>
S×F	4	108230**	19618 <sup>ns</sup>	125839 <sup>ns</sup>	42525 <sup>ns</sup>	0.22 <sup>ns</sup>	0.05**
Error	9	17643	21087	103373	27055	0.16	0.006
CV%		5	12	9	5	16	5

ns: Non-significant, \*\*, \*:significant at 1% and 5% probability level respectively

**Table 6. Mean comparison of salinity levels and ascorbic acid spraying on potassium, sodium and K/Na ratio of leaf and root of St John's wort**

Treatments	Leaf potassium	Root potassium	Leaf sodium	Root sodium	Leaf K/Na Ratio	Root K/Na Ratio
<b>Salinity levels</b>	-----μmol/ gDW-----					
2	840.11 <sup>a*</sup>	454.66 <sup>a</sup>	886.46 <sup>b</sup>	844.66 <sup>b</sup>	0.95 <sup>a</sup>	0.55 <sup>a</sup>
6	892.38 <sup>a</sup>	387.43 <sup>b</sup>	1197.5 <sup>a</sup>	938.21 <sup>ab</sup>	0.75 <sup>b</sup>	0.44 <sup>b</sup>
10	856.48 <sup>a</sup>	360.86 <sup>b</sup>	1273.8 <sup>a</sup>	991.28 <sup>a</sup>	0.80 <sup>b</sup>	0.37 <sup>c</sup>
<b>Ascorbic acid foliar (mg.L<sup>-1</sup>)</b>						
0	850.78 <sup>b</sup>	398.44 <sup>a</sup>	1019.4 <sup>a</sup>	862.07 <sup>b</sup>	0.85 <sup>a</sup>	0.48 <sup>a</sup>
200	907.60 <sup>a</sup>	415.13 <sup>a</sup>	1160.2 <sup>a</sup>	919.57 <sup>ab</sup>	0.90 <sup>a</sup>	0.45 <sup>a</sup>
400	830.60 <sup>b</sup>	389.37 <sup>a</sup>	1179.2 <sup>a</sup>	992.51 <sup>a</sup>	0.76 <sup>a</sup>	0.43 <sup>a</sup>

In each column and for each treatment means with the same letter do not have statistically significant differences at 5% level of probability according to LSD

**Table 7. Results of variance analysis for the effect of salinity and ascorbic acid foliar application on shoot and root dry weight of St John's wort**

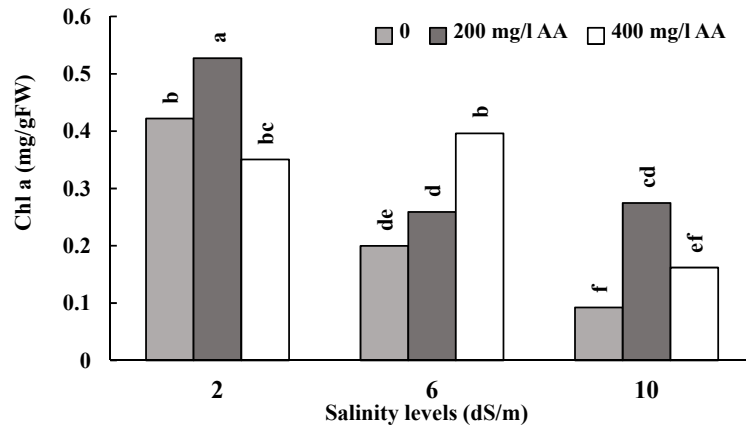
S.O.V	df	Shoot dry weight	Root dry weight
Salinity(S)	2	0.27**	0.14**
Foliar application (F)	2	0.08**	0.07**
S × F	4	0.5**	-0.20**
Error	9	0.01	0.01
CV%		4	5

ns: Non-significant, \*\*, \*:significant at 1% and 5% probability level respectively.

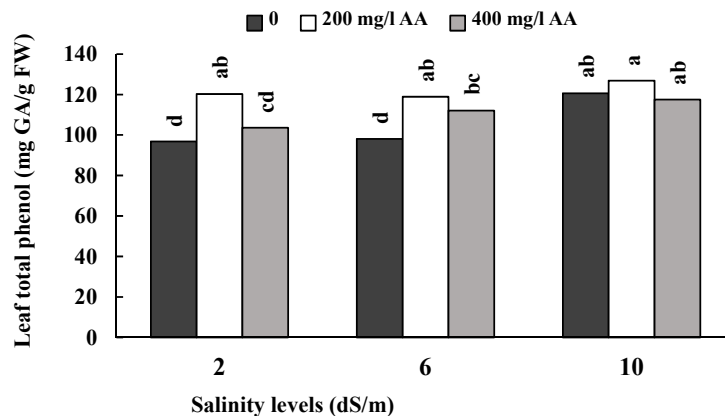
**Table 8. Mean comparison of salinity levels and ascorbic acid foliar application shoot and root dry weight of St John's wort**

Treatments	Shoot dry weight	Root dry weight
----- (g /plant) -----		
<b>Salinity levels (dS.m<sup>-1</sup>)</b>		
2	* 0.97 <sup>a</sup>	0.86 <sup>a</sup>
6	0.72 <sup>b</sup>	0.69 <sup>b</sup>
10	0.70 <sup>b</sup>	0.66 <sup>b</sup>
<b>Ascorbic acid foliar application (mg.l<sup>-1</sup>)</b>		
0	0.71 <sup>b</sup>	0.65 <sup>b</sup>
200	0.82 <sup>a</sup>	0.78 <sup>a</sup>
400	0.87 <sup>a</sup>	0.79 <sup>a</sup>

In each column and for each treatment means with the same letter do not have statistically.



**Fig. 1. Interaction effect of salinity × foliar application of ascorbic acid on Chl a content**



**Fig. 2. Interaction effect of salinity × foliar application of ascorbic acid on leaf total**

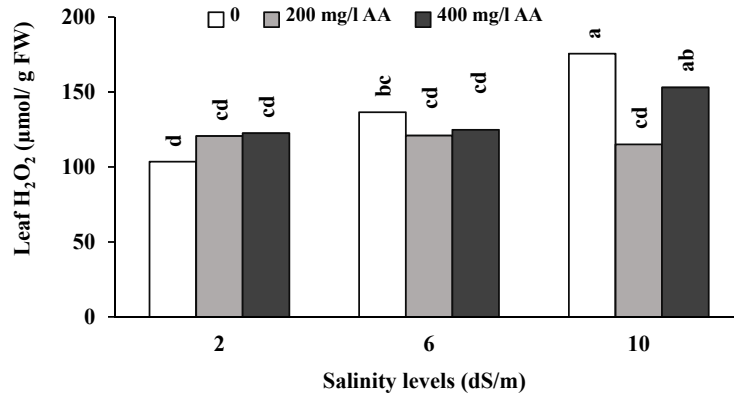


Fig. 3. Interaction effect of salinity × foliar application of ascorbic acid on leaf hydrogen peroxide

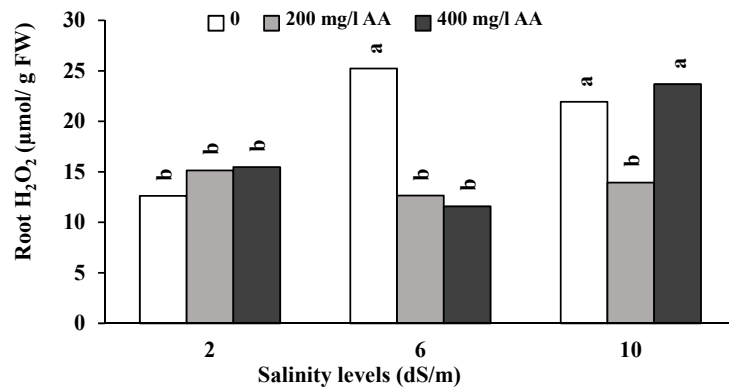


Fig. 4. Interaction effect of salinity × foliar application of ascorbic acid on root hydrogen peroxide

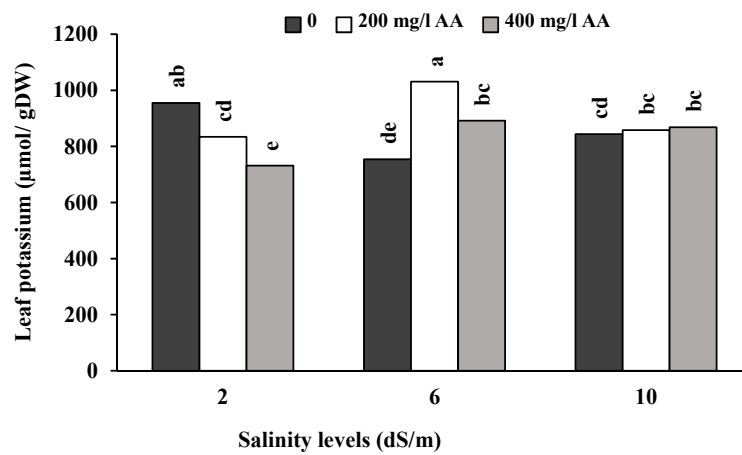


Fig. 5. Interaction effect of salinity × foliar application of ascorbic acid on leaf potassium

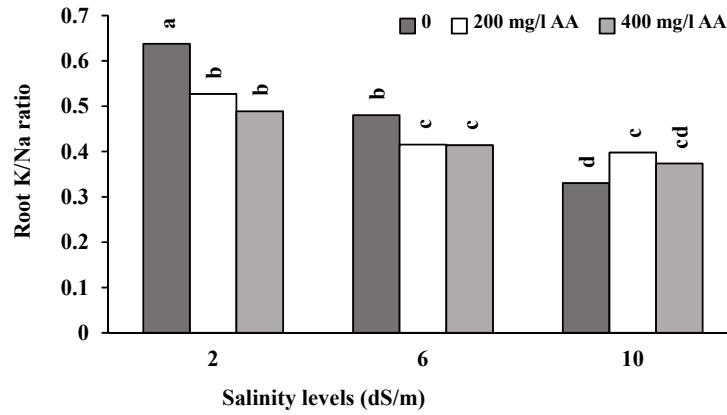


Fig. 6. Interaction effect of salinity × foliar application of ascorbic acid on root K/Na

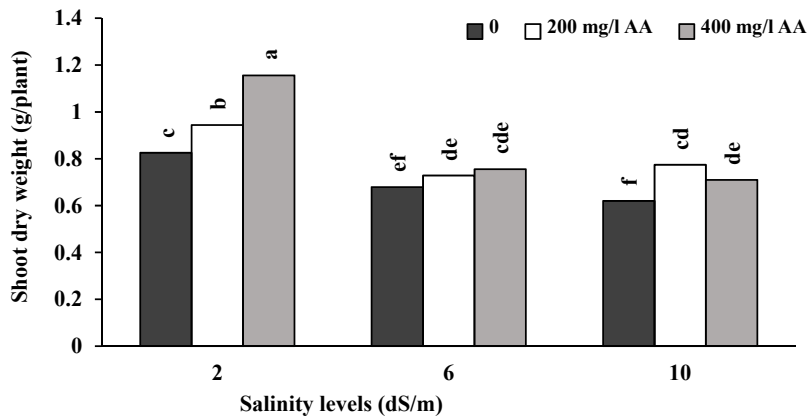


Fig. 7. Interaction effect of salinity × foliar application of ascorbic acid on shoot biomass

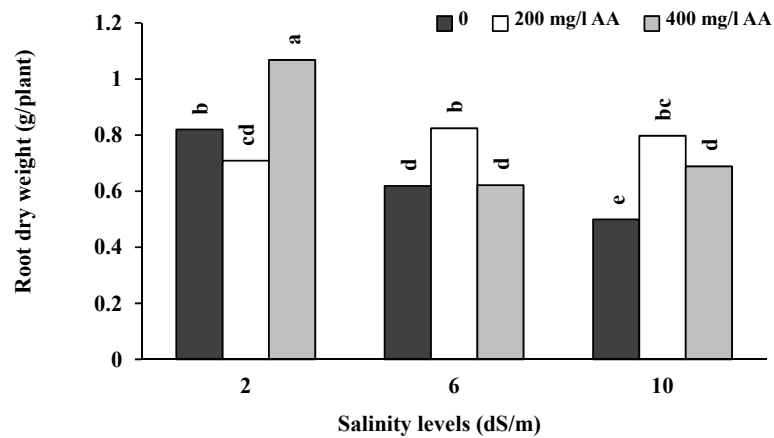


Fig. 8. Interaction effect of salinity × foliar application of ascorbic acid on root biomass